REMARKS

Any fees that may be due in connection with this application throughout its pendency may be charged to Deposit Account No. 50-1213.

Claims 4 and 11-16 are pending. The specification is amended to correct minor typographical and spelling errors. Included as an attachment is a marked-up version of the specification paragraphs, per 37 C.F.R. §1.121. No new matter has been added.

An abstract of the disclosure as required by 37 C.F.R. 1.72(b) is provided on a separate sheet.

A Supplemental Information Disclosure Statement also accompanies this amendment.

Formal drawings are being submitted to the Drawings Review Branch under separate cover.

FINALITY OF OFFICE ACTION

It is respectfully submitted that finality of the Office Action is premature because new grounds of rejections that were not necessitated by amendment were raised. A Petition pursuant to 37 C.F.R. §1.181 to remove the finality of the Office Action was mailed on July 10, 2002.

Claims 4, 11-16

Claims 4 and 11-16 are rejected under 35 U.S.C. §112, first paragraph. It is urged that, specification, while being enabling for the use of specific linkers (npeoc, npc and nps) with specific reactions (deprotection reactions), does not reasonably provide enablement for any type of protecting groups and deprotection reagents. It is further alleged that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. It is stated in the Office Action that Applicant's amendment necessitated the new grounds of rejection. Applicant respectfully traverses this finding for the following reasons.

Claim 4 was rewritten in a previous response, dated August 27, 2001, as an independent claim, incorporating the limitations of the base claims (original claims 1 and 3). The amendments to claim 4 did not add substantive new limitations, but rather merely incorporated the limitations of the base claims 1 and 3. Therefore, to the extent that amended claim 4 is rejected under 35 U.S.C. §112, first paragraph, original claim 4 could have been so-rejected. Therefore the new ground of rejection was not necessitated by amendment.

Similarly, rejection of claims 11-16, which depend from claim 4 and its dependents, under 35 U.S.C. §112, first paragraph, as set forth above also was not necessitated by amendment of the claims.

Claim 15

In the Office Action, claim 15 is rejected under 35 U.S.C. §112, first paragraph, as allegedly containing new matter. The Office Action alleges that "photolytic conditions" claimed in claim 15 has no clear support in the specification and claims as originally filed. It is further alleged that the subject matter claimed in claim 15 broadens the scope of the claims as originally disclosed in the specification. Applicant respectfully traverses this finding.

The amendment to claim 15 mailed on August 27, 2001, only changed the grammatical form, not the substance, of the claim. Therefore to the extent that amended claim 15 is rejected, original claim could have been so-rejected in a previous Office Action. Therefore, the new ground of rejection was not necessitated by the amendment.

THE REJECTION OF CLAIM 15 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claim 15 is rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors(s), at the time the application was filed, had possession of the claimed invention. The Office Action contends that the linkage cleavable under "photolytic conditions" claimed in claim 15 has no clear support in the specification and the claims as

originally filed. It is alleged that the specification discloses the cleavage of linkage between the supports and the monomers by the use of reagents which are acid, base or neutral. It is further alleged that the subject matter claimed in claim 15 broadens the scope of the invention as originally disclosed in the specification. The rejection is respectfully traversed.

RELEVANT LAW

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject mater at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the "written description" requirement is broader than to merely explain how to "make and use"; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the "written description" inquiry, whatever is now claimed." Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64; 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] Vas-Cath, Inc. v. Mahurkar, at 1115, quoting In re Ruschig, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written

description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." Ralston Purina Co. v. Far-Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

Analysis

It is respectfully submitted that claim 15 as originally filed provides basis for the recitation "photocleavable conditions."

Claim 15 as originally filed recited "A process of claim 12, wherein the linkage can be cleaved under acidic, alkaline, neutral or **photolytic conditions.**" Therefore claim 15 as originally filed has support for the linkage cleavable under "photolytic conditions." Claim 15 was amended in a response filed on August 27, 2001, to change grammatical form of the claim. Amended claim 15 reads "A process of claim 12, wherein the linkage is cleavable under acidic, alkaline, neutral or photolytic conditions." Thus the amendment changes the grammatical form and not the substance of the claim and does not add new matter.

Therefore applicant had possession of the claimed subject matter and the application as originally filed discloses the subject matter as it is presently claimed, at the time of filing.

THE REJECTION OF CLAIMS 4, 11-16 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 4, 11-16 are rejected under 35 U.S.C. §112, first paragraph, because the specification while allegedly enabling for the use of specific linkers (npeoc, npc and nps) with specific reactions (deprotection reactions), allegedly does not reasonably provide enablement for any type of protecting groups and

deprotection reagents. It is alleged that the specification does not enable any person in the art to which it pertains, or with which it is mostly connected, to make and use the invention commensurate in scope with these claims because the specification fails to give adequate direction and guidance as to the means of making combinatorial libraries using any type of protecting groups to protect any reactive functional groups using deprotection reagents. The Office Action states that the specification discloses the criterion for selection of deprotection reagents and that the sequence of deprotection reactions are specific or predetermined based on the stability of the protection groups. The Office Action alleges that the working examples are directed to the use of specific protecting groups and deprotection reagents or conditions and the breadth of the claims is open ended regarding the use of protecting groups, deprotection reaction conditions, and the order of the deprotection reactions. It is alleged that the art is inherently unpredictable because the use of protecting groups in a specific position may be unstable during the deprotection conditions, and result in unwanted reactions to occur. The Office Action concludes that it would take undue trials and errors to practice the claimed invention. This rejection is respectfully traversed.

RELAVANT LAW

To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. *Atlas Powder Co. v. E.I. DuPont de Nemours*, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be satisfied by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." *In re Anderson*, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of 35 U.S.C. §112, first paragraph "can be fulfilled by

the use of illustrative examples **or** by broad terminology." *In re Marzocchi et al.*, 469 USPQ 367 (CCPA 1971)(emphasis added).

The inquiry with respect to scope of enablement under 35 U.S.C. § 112, first paragraph, is whether it would require **undue** experimentation to make and use the subject matter as claimed. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims (i.e. the "Forman factors"). *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

PTO GUIDELINES

The standard for determining whether the specification meets the enablement requirement is whether it enables any person skilled in the art to make and use the claimed subject matter without **undue** experimentation. In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400 (Fed. Cir. 1999) (emphasis added). In determining whether any experimentation is "undue," the above-noted factors are to be considered.

As instructed in the published PTO guidelines, it is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above factors while ignoring one or more of the others. The analysis must consider all the evidence related to each of the factors, and any conclusion of non-enablement must be based on the evidence as a whole. *Id.* 8 USPQ2d at 1404 & 1407.

The starting point in an evaluation of whether the enablement requirement is satisfied is an analysis of each claim to determine its scope. As set forth in the guidelines, all questions of enablement are evaluated against **the claimed**

subject matter. The focus of the inquiry is whether everything within the scope of the claim is enabled. With respect scope of enablement, the only relevant concern should be whether the scope of enablement provided to one skilled in the art by the disclosure is commensurate with the scope of protection sought by the claims. *In re Moore*, 439 F.2d 1232, 169 USPQ 236 (CCPA 1971). Once the scope of the claims is addressed, a determination must be made as to whether one skilled in the art is enabled to make and use the entire scope of the claimed invention without undue experimentation.

Analysis

Applying the above factors to the instant claims, applicant respectfully submits that as described in detail below, it would not require undue experimentation to practice the claimed methods.

Scope of the claims

Claim 4, and claims 11-16 dependent thereon, are directed towards a process for generating a combinatorial library, comprising the steps of:

(a) preparing a plurality of immobilized molecules selected from a nucleoside and a nucleotide; wherein each molecule contains 3 to 10 reactive moieties, each reactive moiety being blocked by a blocking group, wherein at least three of the blocking groups on each immobilized molecule are independently removable under at least three different conditions; and

(b) removing each blocking group and derivatizing the resulting reactive moiety in a preprogrammed, regioselective manner; wherein each member of the plurality of immobilized molecules is uniquely derivatized at at least one reactive moiety with a unique substituent, thereby generating a combinatorial library."

Thus the claims are directed towards a process for generating a combinatorial library which is described in the specification in detail by disclosing all the steps involved. Each step of the process is described and

taught in the subject specification (see pages 7-10). The specification, including the working examples, describe in great detail preparation of immobilized oligonucleotides by phosphoramidite method and disclose modifications to this strategy for extension to other oligonucleotide synthesis methods such as phosphotriester method (pages 11, 14-20). Various blocking groups for the reactive moieties in the molecules on phosphate and nucleoside bases are well characterized in the instant application and are well known to those with skill in the art, as are the deprotecting reagents for selective orthogonal deprotection (see pages 12-13). Furthermore the specification discloses stability of various protecting groups under the reaction conditions of orthogonal deprotection and cites a large number of articles, to describe protecting groups for reactive moieties in oligonucleotide synthesis. Therefore the claim 4, and claims 11-16 dependent thereon, are directed towards a process for generating a combinatorial library which is described in the specification.

The level of skill in the art is high

The level of skill in this art is recognized to be high (see, e.g., Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). In addition, the numerous articles and patents that are of record in this application that are authored by those of a high level of skill for an audience of a high level of skill further evidences the high level of skill in this art.

Knowledge of those of skill in the art:

At the time of the effective filing date of this application and before, the skilled artisan knew various protecting groups and deprotection reagents and conditions for use in nucleotide/nucleoside synthesis. Further, there is a large body of literature directed to the use and stability of various protecting groups under different reaction conditions. Selective removal of various protecting groups by using deprotection reagents is also well recorded in the art and known to those of skill in the art.

The articles cited in the specification, of record in this application and

attached hereto describe various protecting groups for reactive functionalities in nucleotide synthesis. For example protection of the carbohydrate hydroxy functions, 5' and/or 3'- hydroxy functions, phosphate protection and protection of the amino function on the bases is discussed in extensive details in the article published by Amarnath *et al.*, Chemical Reviews, **1977**, 77, 183-217. The protecting groups are categorized as acid labile, base labile and groups removable under neutral conditions. The reference describes reagents and conditions for deprotection of the protecting groups, for example 2,4-dinitrobenzenesulfenyl protecting group on 5'-hydroxy site of nucleosides can be removed by thiophenol in phenol.

An extensive review published by E. Sonveaux, *Bioorg. Chem.,* **1986,** 14, 274-325, discusses several protecting groups for use in different oligonucleotide synthesis methods for individual bases and for 3'- and 5'-hydroxy groups.

An article by Reese, C. B., *Tetrahedron,* **1978,** 34, 3143-79, reviews various protecting groups for -OH functionalities and for the bases.

Watkins *et al.* in *J Am. Chem. Soc.,* **1982,** 104, 5702-08, have described use of benzyloxycarbonyl group removable under neutral hydrogenolysis conditions for base protection in oligonucleotide synthesis.

Gioeli *et al.* in *J. Chem. Soc. Chem. Commun.,* **1982,** 672-74, have described Fmoc group removable by basic reagents such as aqueous ammonia, piperidine, ethanolamine or morpholine, in the 5'-*O*-Fmoc-2'-deoxythymidine having orthogonal deprotection properties described in the instant application.

Kharasch *et al. J. Amer. Chem. Soc.,* **1953,** 75, 2658-60, have described 2,4-dinitrophenylsulfenyl (dnps) group in the dnps ethyl ester which reveals selective deprotection properties with deprotection reagents described in the instant application.

Several articles cited in the application on page 13 disclose phosphate and base protection groups and deprotection reagents.

In addition, a large body of publications, not cited in the application, describe protecting groups for nucleoside bases. Some exemplary publications are listed below.

U.S. Patent Nos. 5,763,599 and 5,652,358, describe phenoxyacetyl, benzoyl, isobutyroyl, p-(t-butyl)benzoyl and p-(t-butyl)phenylacetyl protecting group for nucleotide bases.

Koster et al., Tetrahedron 37, 363-369, and Ti *et al.* J. Am. Chem. Soc. 1982, 104: 1316-1319, report several acyl protecting groups for use in oligonucleotide synthesis. Comparative rates of deacylation of various acyl protecting groups in MeOH/NaOH mixture are also reported.

Rasmussen *et al.* J. Am. Chem. Soc. 1967, 89(21): 5439-45, disclose pivaloyloxymethyl protecting group removable under mildly basic conditions, for adenine.

Hayakawa *et al*. J. Am. Chem. Soc. 1990, 112: 1691-1696, describe allyloxycarbonyl (AOC) protecting group for nucleoside bases. AOC group can be removed by palladium(O) catalyzed reaction under mild conditions.

Vu *et al.* Tetrahedron Letters, 1990, 31, 7269-7272, describe dialkylformamidine and isobutyryl protection of nucleosides. Deprotection can be achieved under mild basic conditions.

Dreef-Tromp *et al.* Tetrahedron Letters, 1990, 31, 427-430, describe 2-(*tert*-butyldiphenylsilyloxymethyl)benzoyl protecting group removable under neutral conditions by fluoride ion.

U.S. Patent No. 5,614,622 describe the use of 5-Pentenoyl moiety as nucleoside amino protecting group in oligonucleotide synthesis. It can be deprotected by chemoselective removing agents for example, halogens in water or pyridine/alcohol or by nonchemoselective removing agents such as aqueous ammonium hydroxide or alcoholic ammonia.

Caruthers *et al*. Nucleosides & Nucleotides, 4(1&2), 95-105, have described various amidine protecting groups which can be removed under basic

condition, for nucleoside bases.

Letsinger *et al.* J. Am. Chem. Soc. 1969, 91:12: 3356-59, describe β-benzoylpropionyl and benzoylformyl for -OH protection of nucleosides and isobutyloxycarbonyl for -NH_2 protection during oligonucleotide synthesis. These can be removed under neutral conditions.

Vinogradov *et al.* Tetrahedron Letters 1993, 34, 5899-5902, describe isopropoxyacetal group for the protection of the exocyclic amine of the nucleic bases. Deprotection was achieved under basic conditions.

Kamimura *et al.* Tetrahedron Letters 1983, 24, 2775-2778, reported diphenylcarbamoyl group for protection of 6-O and propionyl group for protection on amino group in guanine. It was removed by ammonia + pyridine.

McBride *et al.* Tetrahedron Letters 1983, 24, 2953-56, reported N-methyl-2-Pyrrolidine amidine group as deoxynucleoside protecting group and removal was achieved by ethylenediamine:phenol.

Ogilvie *et al.* Tetrahedron Letters 1982, 23, 2615-18, describe Levulinyl group for amino protection in nucleosides and hydrazine as deprotection reagent.

Froehler *et al.* Nucleic Acid Research 1983, 11, have reported dialkylformamidine protecting group removable with ammonia, for N protection in deoxyadenosine.

Hence, those of skill in the art are well- aware of various protection groups and deprotection reagents. Based on the teachings and guidance in specification and the knowledge of those of skill in the art, one can readily select those groups and reagents that meet the criteria for selective orthogonal deprotection for making combinatorial libraries per the instant claims.

The amount of direction and guidance presented, teachings in the specification and presence of working examples

The specification describes synthesis of oligomers for sequence specific selective and orthogonal deprotections and for subsequent derivatizations by the use of differently base and/or phosphate protected building blocks (Scheme 1,

page 7-8). The specification describes and exemplifies various phosphate and base protecting groups and their stability during deprotection reactions (see page 13, lines 7-21, pages 14-15, lines 20-10, pages 19-20, lines 13-15, page 24, lines 1-15). Numerous articles cited in the application teach the use of various protecting groups for protection of reactive functionalities in solid phase synthesis of combinatorial libraries and deprotection reagents and conditions. The working examples provided exemplify multiselective deprotection via sequence dependent preprogrammed selection of appropriate nucleotide building blocks to create oligomers with predetermined modifications, various combinations of which can generate a combinatorial set of molecules.

Conclusion

In light of the scope of the claims, the teachings in the specification, the high level of skill of those in this art, and the extensive knowledge of those of skill in this art, it would not require undue experimentation for a person of skill in the art to select blocking groups to block reactive moieties wherein at least 3 blocking groups are removable under at least 3 different conditions that are within scope of the instant claims, and subsequently derivatize the reactive moieties to generate the claimed combinatorial libraries of nucleotides or nucleosides. Therefore, the specification is enabling for making and using the full scope of the claimed subject matter .

Rebuttal to Arguments in the Office Action

The Office Action alleges that the specification fails to give adequate direction and guidance as to the means of making combinatorial libraries using any type of protecting groups to protect any reactive functional groups using deprotection reagents. It is respectfully submitted that a process for generating a combinatorial library is described in the specification including examples in detail by disclosing all the steps involved. As discussed above, the specification teaches preparation of plurality of immobilized molecules selected from a nucleoside and a nucleotide, use of various protecting groups for the reactive

moieties in the molecules on phosphate and nucleoside bases is well characterized and exemplified, as is the use of deprotecting reagents and conditions for selective orthogonal deprotection. Furthermore, one of skill in the art, based on the references cited in the specification and provided herein, would know how to select other protecting groups within the scope of the instant claims because the specification sets forth the criteria for selection of protecting groups and deprotection reagents under reaction conditions of claimed combinatorial libraries. Therefore the specification provides adequate direction and guidance to make the combinatorial libraries per the instant claims.

The Office Action alleges that the claims are open ended regarding the use of protecting groups and deprotection reaction conditions, and the order of the deprotection reactions. It is respectfully submitted that the claims at issue are directed to a process for generating combinatorial library and one of skill in the art would know that to make a combinatorial library various combinations of protection and deprotection reactions are used. All the possible combinations are contemplated to be within the scope of claims. The specification discloses stability of various protecting groups under the reaction conditions of orthogonal deprotection and sets forth the criteria for selection of protecting groups for reactive moieties in oligonucleotide synthesis. Furthermore various protecting groups and deprotection reaction conditions used in oligonucleotide synthesis are described in detail in the specification and are known to those of skill in the art, as evidenced by the references cited in the application and listed above.

Applicant is entitled to claims that are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed. In the above-captioned application, Applicant describes exemplary protecting groups for reactive moieties in the molecules on phosphate and nucleoside bases for use in the synthesis of claimed combinatorial library. Based on the reaction conditions of orthogonal deprotection disclosed explicitly

in the application and the information about various protecting groups available in the art, one of skill in the art can select appropriate protecting groups and deprotection reaction conditions for use in generation of combinatorial library commensurate with the claims.

The Office Action further alleges that the art is inherently unpredictable because the use of protecting groups in a specific position may be unstable during the deprotection conditions, and result in unwanted reactions to occur. Applicants respectfully disagree. Use of protection/ deprotection strategy in oligonucleotide synthesis is extensively documented in art and has been in use for many years, as evidenced by number of articles cited in the instant application and in this response. Problems encountered in the use of specific protection groups under specific deprotection conditions are predictable because the stability of protection groups under various reaction conditions for use in synthesis of combinatorial libraries has been extensively studied and well documented in the art. Therefore, a person of skill in the art could readily select appropriate protecting groups under the reactions conditions of selective orthogonal deprotection described in the instant application for use in generation of a combinatorial library commensurate with the claims.

* * *

In view of the remarks herein, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Köster et al.

Serial No.: 09/171,625

Filed: July 2, 1999

For: A COMBINATORIAL PROTECTING GROUP STRATEGY FOR MULTIFUNCTIONAL MOLECULES

Art Unit: 1627

Examiner: Ponnaluri, P.

ATTACHMENT TO THE AMENDMENT
MARKED UP PARAGRAPHS (37 CFR §1.121)

IN THE SPECIFICATION:

Please amend the specification as follows:

Please amend the paragraph beginning on page 1, line 25, through the paragraph on page 2, line 7, as follows:

H-phosphonate or [phosphoamidite] phosphoramidite chemistries employing solid phase methods in automated DNA synthesizers are most efficient for the synthesis of oligonucleotides. The [phosphoamidite] phosphoramidite method using B-cyanoethyl [phosphoamidites] phosphoramidites as reactive nucleotide building blocks is the most prevalent synthesis method due to the quantitative condensation yields despite an oxidation step in every cycle (Sinha, N.D. *et al., Tetrahedron Lett.,* 1983, 24, 5843-46; Sinha, N.D. et al., Nucleic Acids *Res.,* 1984, 125 4539-57; Froehler, B.C. et al.*, Nucleic Acids* Res., 1984, 14, 5399-5407; Froehler, B.C. and Matteuci, M.D., *Tetrahedron Lett.*, 1986, 27, 469-72; Garegg, P.J. *et al., Tetrahedron Lett.,* 1986, 27, 4051-54; Sonveaux, E., *Bioorg. Chem,*, 1986, 14, 274-325; Uhimann, E. and Peyman, A., *Chem. Rev.*, 1990, 90, 543-84).

Please amend the paragraph on page 9, lines 3-14, as follows:

As shown in scheme 1, a 2'-deoxyoligonucleotide, **3,** is synthesized, e.g. by the [phosphoamidite] phosphoramidite method (Sinha, N.D., Biernat, J.,

Köster, H. *Tetrahedron Lett.,* **1983**, 24, 584346; Sinha, N.D., Biernat, J., McManus, J., Köster, H. *Nucleic Acids* Res., **1984**, 12, 4539-57; Sonveaux, E. *Bioorg. Chem.,* **1986**, 14, 274-325). However, in contrast to the usual 3′ to 5′ addition, the synthesis is performed in the 5′ to 3′ direction using the building blocks **1** and **2**. During an elongation cycle, the temporary protecting group, R^3, is removed, e.g. using a neutral hydrazine reagent IV (table 1) before the condensation step and the acidified filtrate of the hydrazinolysis solution is spectrophotometrically measured to determine the preceding condensation yield. In this manner, a trityl assay as typically used with the 4, 4′-dimethoxytrityl group, is possible. In addition, there is little risk of depurination, since acidic conditions are not used during the synthesis cycles.

Please amend the paragraph beginning on page 11, line 8, through the paragraph on page 12, line 2, as follows:

The selective and orthogonal deprotections and the derivatizations by introducing new substituents can be carried out at positions ①-④, at ② and ③, in a *sequence specific* way. During the derivatizations at ①-④ only the npeoc/npe base protection remains intact. In contrast, the phosphate protecting group R^{4B} needs to remain intact if derivatizations at ② are to be performed. These two protecting groups only serve to carry out *sequence specific* derivatizations at ② and/or ③. *After* the derivatizations at least the bases, protected with npeoc/npe groups have to be deprotected without removing new substituents at ①-④ at the same time. The removal of the npeoc/npe groups is necessary to guarantee sufficient hybridization properties of the [derivatizated] <u>derivatized</u> oligomers with complementary nucleic acid sequences.

Please amend the paragraph on page 14, lines 14-19, as follows:

Compared to current oligodeoxynucleotide syntheses for use in antisense and triplex DNA therapies (Cohen, J.S., Hogan, M.E., *Scientific American, Int. Ed.*, December **1994**, pages 50-5514; Uhlmann, E., Peyman, A., *Chem Rev.*, **1990**, 90, 543-84; Beaucage, S.L., Iyer, r.P. Tetrahedron, **1993**, 49, 6123-94), the new strategy shows a remarkable advantage. All possible [derivitizations]

derivatizations can be performed with only *one* oligonucleotide synthesis run.

Please amend the paragraph beginning on page 14, line 20, through page 15, line 10, as follows:

The strategy presented above, can be modified according to other oligonucleotide synthesis schemes. For example, in addition to the phosphoramidite method shown in scheme 1, the strategy can be employed with the phosphotriester and other suitable methods of oligonucleotide synthesis. For the phosphotriester method, chloro substituted phenyl groups and the ß-cyanoethyl group were successfully used as phosphate protection groups (Amarnath, V., Broom, A. D.. *Chem. Rev.* **1977**, 77, 183-217; Reese, C. B., *Tetrahedron,* **1978,** 34, 3143-79). The levulinic acid ester and the npeoc/npe base protection are stable during the reaction conditions of the phosphotriester method (Himmelsbach, F., Schulz, B.S., Trichtinger, T., Ramamurthy, C., Pfleiderer, W., *Tetrahedron,* **1984,** 40, 59-72; van Boom, J.H., Burgers, P.M.J., *Tetrahedron Lett.,* **1976,** 4875-78). The nps base protection has been successfully used during the oligonucleotide synthesis by the phosphotriester approach (Heikkila, J., Balgobin, N., Chattopadhyaya. J., *Acad Chem. Sci.,* **1983,** B37, 857-62). The structure of oligomers obtained in this way of synthesis is the same as for the oligomer **3** generated by the [phosphoamidite] phosphoramidite method (scheme 1). For syntheses by the [phosphoamidite] phosphoramidite method, amidites, whose 5'-OH or 3'-OH groups respectively are protected with the 4,4'-dimethoxytrityl (DMTR) group, are used. Scheme shows a general view and scheme 4 to 6 show specific examples.

Please amend the paragraph on page 26, lines 4-14, as follows:

a: protection of compound **42** with **39** (scheme 8), **32** (scheme 7)[1]),),4-dinitro- or 2-nitrophenylsulfenyl chloride b: aminolysis with aminopropyl-CPG, followed by reaction with Fmoc chloride [c:orthogonal] c: orthogonal deprotections of the Fmoc (9-fluorenylmethoxycarbonyl) group and group R^6 and derivatizations with new substituents R^8 and R^9 respectively, orthogonal

deprotection of the levulinic ester moiety d: reaction with succinic anhydride e: reaction of compound **46** with **47.** Compound **47** is (as compound **46)** a derivative of compound **44** but *otherwise derivatized* (with new substituents R^{10} and R^{11} in compound **47** in contrast to R^8 and R^9 in **46**). **47** was removed from the support. The reaction to **48** can be carried out as described by Gupta, K.C. *et al.*, *Nucl. Acids Res.*, **1991**, 19, 3019-25. The [succesfull] successfull reaction to **48** can be monitored by vis spectroscopy of **48** after treatment with acid.

Please amend the paragraph on page 27, lines 4-23, as follows:

The structure of the protecting groups is very useful for the reaction control by thin layer chromatography (tlc) during the synthesis of compound **43.** Each reaction step can be controlled by a specific colorimetric effect and UV-detection. This is demonstrated by the following description. If a compound **42** with e.g. four hydroxyl groups is monosubstituted by compound **32** (scheme 7), treatment with acid leads to an orange product (trityl cation), but the colorimetric trityl moiety is not cleaved. After the second monosubstitution with **39** (scheme 8), detection with acid leads to two orange products, because one of the trityl moieties is now cleaved off. Additionally, intensive yellow [colour] color can be observed by ammonia vapour (or by primary and secondary amines), due to released p-nitrophenolate ions. The product obtained after the third monosubstitution with dnps chloride already shows yellow [colour] color without any detection reagent (and of course the other colorimetric effects). Protection of the last free hydroxyl group with Fmoc chloride should be done *after* the reaction of compound **43** with aminopropyl CPG, because of the sensitivity of Fmoc esters in the presence of amino groups. Nevertheless, the last free hydroxyl group of a sample of compound **43** can be substituted by a nucleoside derivative (the reactive form of 5'-*O*-DMTr-T_d-*O*3'-succinic mono ester e.g.). By contact with sugar spray reagent and heating with a fan an *additional* green [colour] colored product can be observed on tlc (due to the superposition of the blue [colour] color of the nucleoside and the orange [colour]

color of the trityl moieties). This shows the possibility to control four successive monosubstitions by different colorimetric effects.

Please amend the paragraph beginning on page 28, line 16, through the paragraph on page 29, line 24, as follows:

^{1}H (400 and 250 MHz) and ^{13}C)101 and 63 MHz) NMR spectra were recorded on a Bruker AMX 400 and a AC 250-P instrument. Samples were dissolved in the presence of tetramethylsilane as internal standard, unless otherwise stated. ^{31}P NMR [sprectra] spectra were [recordered] recorded on a Varian Gemini 200 instrument. Internal standard: phosphoric acid in the solvent used for the sample (δ = 0.00 ppm), Chemical shifts are given in ppm. Mass spectra were obtained on a Finnigan MAT 311A mass spectrometer under EI conditions, a VG Analytical 70-250S mass spectrometer under FAB conditions (matrix: 3-nitro-benzyl alcohol, Xenon bombardment) and a Finnigan MAT Vision 2000 mass spectrometer under MALDI-TOF conditions (matrix solution: 0.7 mol/ 13-hydroxy picolinic acid and 0.07 mol/ 1 ammonium citrate in acetonitrile/ water, 1/1, v/v). Elementary analyses were performed by the analytical department of the Institute of Organic Chemistry, University of Hamburg. Thin layer chromatography (tlc) was carried out on 60 PF_{254} silica gel coated alumina sheets (Merck, Darmstadt, No 5562). Trityl and sugar containing compounds are visualized with sugar spray reagent (0.5 ml 4-methoxybenzaldehyde, 9 ml ethanol, 0.5 ml concentrated sulfuric acid and 0.1 ml glacial acetic acid) by heating with a fan or on a hot plate. p-Nitrophenyl ester containing compounds are visualized by ammonia vapour. Column chromatography was performed using silica gel from Merck. HPLC results were obtained on a Waters chromatography systems 625 LC with a photodiodearray detector 996 and using reversed phase columns (Waters Nova-Pak C18, 60 Å, 4 μm particles, 3.9 x 300mm, software: Millenium 2.0, eluants were: 0.1 M triethylammonium acetate at pH 7.0 (A) and acetonitrile (B); the column was equilibrated at 30°C at 1ml per min, with 95% A/ 5% B, v/v, with elution using a linear gradient from 5% to 40% B in 40 min, monitored at 254 nm). Spectrophotometric

measurements in the UV/ Vis region were performed on a Beckman UV35 and a LKB Ultrospec Plus UV/ Vis spectrophotometer. Solvents were dried and purified before use according to standard procedures. Extractions were monitored by tlc to optimize completion of extraction.

Please replace the paragraph on page 30, lines 2-24, with the following paragraph:

Compound **32** was prepared *in situ* by reacting levulinic acid derivate **31** (Leikauf, E., Köster. H., *Tetrahedron,* **1995,** 51, 5557-62) (3.78 g, 8.39 mmol) with N,N'dicyclohexylcarbodiimide (1.80 g, 8.74 mmol) in dry dioxane (25 ml). N,N'-dicyclohexylurea is removed by filtration and washed with dioxane. The solution was divided in four equal parts and the solvents were evaporated *in vacuo.* To each of the four residues of anhydride **32** was added one of the four following protected nucleosides: 5'-*O*-DMTr-2'-deoxythymidine, 5'-*O*-DMTr-N^4-npeoc-2'-deoxycytidine, 5'-*O*-DMTr-N^6-npeoc-2'-deoxyadenosine, 5'-*O*-DMTr-N^2-npeoc-O^6npe-2'-deoxyguanosine (1.00 mmol of each; base protected deoxynucleosides were from Chemogen, Konstanz) (Stengele, K.P., Pfleiderer, W., *Tetrahedron* Lett., **1990,** 31, 2549-52) and 4-dimethylaminopyridine (0.0100 g, 0.0819 mmol) in 1.64 ml pyridine. Completion of reaction was checked by thin layer chromatography. 30 min after the addition of a mixture of 0.130 ml of glacial acetic acid and 0.245 ml pyridine, 0.046 ml water were added, 60 min later an excess of ethyl acetate was added, the N,N'-dicyclohexylurea removed by filtration and washed with ethyl acetate. The mixture was extracted with water, 5% aqueous sodium hydrogen carbonate and water. After drying with sodium sulfate, the solvent was evaporated, then co-evaporated with toluene. The residues were directly detritylated with 80% acetic acid and the reaction was monitored by thin layer chromatography. The solutions were poured into an excess of water (about [10fold] 10 fold) and the aqueous mixtures were extracted with ethyl acetate. The organic phase was washed with 5% aqueous sodium hydrogen carbonate and water. After drying, the solvent was evaporated, then co-evaporated with toluene (to remove

remaining acetic acid). The residues were directly methylated by adding to each a solution of 200 ml methanol and 1 ml glacial acetic acid. If there were some insoluble material, it was dissolved in 5-10 ml dichloromethane and a mixture of 100 ml methanol and 0.5 ml glacial acetic acid was added. Monitoring by thin layer chromatography indicates completion of the reaction. The solvents were evaporated under reduced pressure, followed by co-evaporation with toluene (2-3 times). The residues of **30a-d** were purified by silica gel column chromatography (**30a**: silica gel 60H, No. 7736, **30b-d**: silica gel 60, No. 9385; Merck, Darmstadt). Silica gel used per gram raw product: **30a**: 25 g, **30b**: 51 g, **30c**: 65 g, **30d**: 51 g; using a step gradient from dichloromethane to dichloromethane/ methanol 98/2 (v/v), in the presence of 0.1% pyridine. Pure fractions were pooled, the solvents removed by evaporation, the residues dissolved in dichloromethane (15 ml per gram residue) and the solutions precipitated into hexane (315 ml per gram residue). Yields: **30a**: 68%, **30b**: 63%, **30c**: 62%, **30d**: 52%.

Please amend the paragraph beginning on page 34, line 24, through the paragrapg on page 35, line 22, as follows:

All steps were carried out under inert [athmosphere] <u>atmosphere</u> (argon). Organic solvents were free from water and other impurities. Compounds **30a-d** (0.5 mmol of each) were azeotropically dried with small amounts of pyridine and toluene and dissolved in 2.43 ml ethyl acetate. After the addition of N,N-diisopropyl ethylamine (1.75 mmol, 0.226 g, corresponding to 0.30 ml at room temperature) the reaction flask was capped with a septum and cooled with an ice bath. Chloro-β-cyanoethoxy-N,N-diisopropylaminophosphane (0.610 mmol, 0.144 g, corresponding to 0.117 ml at room temperature, Biosyntech, Hamburg) was added dropwise by a syringe. 15 min later the reaction was allowed to raise to room temperature. Monitoring by thin layer chromatography (about 60 min after starting the reaction) indicated complete conversions to the amidites **2a-d**. The precipitated amine hydrochloride was filtered off using a column type reactor fitted with a sintered glass fritt and washed with 1.5 ml ethyl acetate.

The solution was extracted in a separation funnel with cold 5% sodium hydrogen carbonate (2 x 2.8 ml). The organic solution was filtered using the described reactor which contains sodium sulfate, followed by washing of the sodium sulfate layer with ethyl acetate (2 x 1.8 ml). After evaporation of the solvents of the filtrate, a foam was obtained. The amidite was dissolved in 5 ml ethyl acetate (containing 0.1% pyridine) and precipitated into 120 ml of hexane (at -20°C). After filtration using the described reactor the amidite was washed with 12 ml of hexane, dried and stored at 20°C. Yields: **2a**: 86%, 2b: 72%, 2c: 78%, 2d: 80%. - ^{31}*P NMR* (81 MHz, CD_3CN/ CH_3CN, I/I,v/v and a trace of N,N-diisopropyl ethylamine): **2a:** δ = 149.18, 149.35 (diastereomers), **2b:** δ = 149.25, **2c:** δ = 149.07, **2d:** δ = 148.89, 149.16 (diastereomers).

Please amend the paragraph on page 36, lines 4-29, as follows:

Compound 35 (25.7 g, 144 mmol) and methoxybenzene (36.8 g, 340 mmol) were stirred in 450 ml glacial acetic acid to dissolve most of the material. The mixture was cooled in an ice bath and immediately concentrated sulfuric acid (225 g, 2290 mmol) added dropwise. The reaction mixture was then stirred at room temperature until thin layer chromatography (dichloromethane/methanol: 8/2, v/v) demonstrated quantitative conversion. The reaction mixture was poured into 3/1 ice/ water. Subsequently the reaction flask was washed with ether and the ether solution was poured into the ice/water. The orange-white raw product between the aqueous and organic layer was filtrated by suction (if there was still a considerable amount of the raw product under the aqueous and/or dissolved in the ether layer, it was also worked up). The raw product was triturated with 200 ml water, filtrated by suction, again triturated with petroleum ether (bp 60-70° C) and filtrated. It was recrystallized from ether. Yield: 22.3 g (41%). Note: More product **36** can be purified from the crystalline residue of the [the] mother liquor by silica gel column chromatography or by Soxhlet extraction with petroleum ether (bp 30-50°C). ^{1}*H NMR* (250 MHz, $CDCl_3$): δ = 2.65 (t, 2H, -$\mathbf{CH_2}$-CH_2-), 2.92 (t, 2H, -CH_2--$\mathbf{CH_2}$-),3.78 (s, 6H, -OCH_3), 5.40 (s, 1H, R_3C-**H**), 7.13-6.77 (m, 12H, aryl-

H). - *^{13}C NMR* (63 MHz, $CDCl_3$, internal standard $CDCl_3$ at 77.00 ppm): δ = 30.1 (t, -**C**H_2-CH_2-), 35.48 (t, -CH_2-**C**H_2-), 54.8, 55.19 (q, aryl-O**C**H_3 and d, R_3**C**-H, position not defined), 113.63, 128.1, 129.41, 130.21 (d, **C**H, aryl), 136.46, 137.91, 142.7 (s, aryl, quartenary), 157.93 (s, R_2**C**-OCH_3, aryl), 178.85 (s, --**C**OOH).- *MS* (EI): m/z (rel. intensity): m/z calculated for $C_{24}H_{24}O_4$ (M^+): 376; found: 376 (100), 345 (9, M-OCH_3 $^+$), 227 (35, M - HOOC-CH_2-CH_2-C_6H_4 $^+$). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{24}H_{24}O_4$ (M^+): 376; found: 376 (48), 345 (8, M-OCH_3 $^+$), 269 (53, M - C_6H_4-OCH_3 $^+$), 227 (38, M - HOOC-CH_2-CH_2-C_6H_4 $^+$). - *Elementary Analysis* (%): Found: C, 76.55/ 76.35; H, 6.71/6.53;$C_{24}H_{24}O_4$ requires C, 76.57; H, 6.43.

Please amend the paragraph beginning on page 40, line 22, through the paragraph on page 41, line 7, as follows:

Compound **40** (0.160 g, 0.135 mmol) was dissolved in dry dioxane (0.311 ml) and dry pyridine (0.032 ml). A suspension of aminopropyl CPG (0.405 g, CPG-10-500, Biosyntech, Hamburg) in 1.27 ml dry N,N-dimethylformamide and 0.160 ml (0.116 g, 1.15 mmol) dry triethylamine was added and the suspension shaken during 21.5 h. An intensive yellow [colour] <u>color</u> indicated beginning reaction caused by released p-nitrophenolate ions. The suspension was shaken during 21.5 h. A ninhydrin test at this stage indicated the existence of free amino groups on the support. To acylate, "cap", these groups, dry triethylamine (0.030 ml) and acetic anhydride (0.090 ml) were added and the suspension was shaken for another 60 min. After this time a negative ninhydrin test was obtained. The support was washed successively with N,N-dimethylformamide, ethanol, dioxane, ether (100 ml each) and dried *in vacuo.* Analysis for the extent of 3'-OH protected nucleoside attached to the support was done spectrophotometrically. An accurately weighed sample was treated either with 5% dichloroacetic acid in dichloromethane (v/v) or with hydrazine reagent IV (table 1) followed by acidifying the solution with 40% trichloroacetic acid in dichloromethane (percentage by weight). The liquid phase was measured at 513 nm (extinction coefficient of an acid solution of the

removed trityl derivate: $\varepsilon = 78600$). Amount of nucleoside bound to the support 1:45.6 μmol/g.

Please amend the paragraph on page 42, lines 8-11, as follows:

6) *Hydrazine reagent*: 0.5M hydrazine reagent IVb [table 1).Reagent] <u>(table 1). Reagent</u> of high quality have to be used: [bidestilled] <u>bidistilled</u> water, acetic acid p.a. (Merck, Darmstadt No. 63), hydrazinium hydrate (Merck, Darmstadt No. 804608), pyridine p.a. (Merck, Darmstadt No. 7463).

Please amend the paragraph on page 44, lines 4-12, as follows:

The support with the attached oligomer was washed with pyridine and the β-cyanoethyl groups were removed with *tert*-butyl amine reagent II (table 1). After washing the support with pyridine and acetonitrile and drying *in vacuo,* the oligomer was removed from the support by treating it with 80% acetic acid for 15 min. After [liphylisation] <u>lyophilization</u> of the solution, the oligomer was purified by HPLC: the terminal 3'-OH protecting group (corresponding to the group of compound **29a** in scheme 7) served here as purification handle. Treatment with 32% ammonia followed by lyophilisation led to the fully deprotected oligomer d(TTTT). - *HPLC:* Ret. time (min): 8.57, UV detection: λmax = 266.1 and 217.7 nm. - MS (MALDI-TOF): theoretical mass: M+H +: 1155; found: 1154.